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# Improved signal fidelity in 4-pulse DEER with Gaussian pulses

Markus Teucher, Enrica Bordignon *

*Faculty of Chemistry and Biochemistry, Ruhr University Bochum, Universitaetsstr. 150, 44801 Bochum, Germany*

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The introduction of arbitrary waveform generator (AWG) technology and the availability of high power microwave amplifiers mark a "new era" in pulse EPR due to significant sensitivity improvements and the possibility to perform novel types of experiments. We present an optimized 4-pulse DEER setup that uses Gaussian observer pulses (GaussDEER) in connection with a Gaussian/shaped pump pulse. Gaussian pulses allow to experimentally remove the "2+1" pulse train ESE signal which is intrinsically present in any DEER experiment performed with rectangular pulses. Further signal improvements are obtained with shaped pump pulses, which can significantly increase the modulation depth of the DEER experiment due to their tailored excitation bandwidth. Although sequences like CP (Carr-Purcell) DEER offer advantages such as a prolongation of the dipolar evolution time, they suffer from post-processing of the time-domain data to remove artifacts. Therefore, it is worth having a 4-pulse DEER experiment free of residual "2+1" signal since this is still the main dipolar spectroscopic technique used in structural biology. In this work we focus on nitroxides, which are the spin probes primarily used in site-directed spin labeling studies of biomolecules, however, the advantages introduced by Gaussian pulses can be extended to any spin type.



## 1. Introduction

Double Electron-Electron Resonance (DEER, also known as PELDOR) [1–3] in combination with site-directed spin labeling is a versatile EPR-technique for monitoring structural domains and conformational changes in proteins. The transition from experiments on proteins in solution towards more physiological conditions, such as proteins embedded in lipid bilayers and in cellular environments still poses some challenges, especially for distances >5 nm, mainly due to low protein concentrations and short relaxation times. The key to approach these challenges lies in the improvement of sensitivity and accuracy of the technique. In this work, we tackle the problem of the "2+1" residual signal in DEER traces and how it can be removed by using Gaussian pulses. Advantages in terms of increased selectivity and suppression of side bands using Gaussian pulses were early recognized [4], but not yet implemented in DEER. We will address setup optimization and the additional benefits created by the use of other shaped pump pulses.

*Sensitivity.* In pulsed EPR, assuming that experiments can be performed in different frequency bands under the same conditions (constant spin number and available power), the sensitivity is proportional to the square of the resonance frequency [5]. Unfortunately, the applicability of this law to reality is challenged by some severe technical limitations. First, the available microwave power decreases at higher microwave frequency bands due to increasing technical difficulties in providing high power amplification. Second, the indirect proportionality between microwave frequency and resonator size leads to decreasing dimensions of the resonator and in turn smaller sample volumes. Furthermore, the spectral density of many spin 1/2 labels (e.g. nitroxides, $Cu^{2+}$) decreases at high frequencies due to the increasing spectral width caused by the Zeeman interaction. The situation is different for high spin systems, such as the spin 7/2 $Gd^{3+}$ labels, for which narrower lines are found at higher frequencies due to the decreased relevance of zero field splitting (ZFS) with respect to the Zeeman interaction. In connection with less available microwave power, the ratio between the fraction of the spectrum that can be excited and the total width of the spectrum becomes more and more unfavorable for S = 1/2 systems, which results in a lower sensitivity and, in case of DEER, possibly, in orientation selection artifacts [6]. The best compromise between all of these factors for DEER performed on nitroxide-labeled samples is currently achieved at Q band (≈34 GHz) [6], while for $Gd^{3+}$ labels W-band frequencies are best suited, if high microwave power and dual mode resonators are available [7,8]. Besides DEER, many different techniques are employed for interspin distance determination, e.g. DQC [9],

* Corresponding author.
*E-mail address:* enrica.bordignon@rub.de (E. Bordignon).

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SIFTER [10], RIDME [11] on various different spin types such as $Cu^{2+}$, $Mn^{2+}$, $Gd^{3+}$, nitroxide and trityl. However, in this work, we will focus merely on DEER experiments performed on nitroxide-containing samples, and discuss the advantages of using Gaussian pulses created by arbitrary waveform generators (AWGs). Nitroxide spin labels are still the main type of probes used for in vitro studies because in contrast to other spin types, they allow detection of side chain dynamics, water accessibility [12] and interspin distances with high accuracy [13], also for in-cell applications [14]. Additionally, DEER is the technique of choice for determination of interspin distances in biomolecules. The main conclusions of this paper, namely that Gaussian pulses increase signal fidelity in DEER can be, however, extended to any type of spin.

*Arbitrary waveform generators.* Novel arbitrary waveform generator technology introduced to EPR by the groups of Prisner and Jeschke [15,16] provides for the first time the possibility to independently tune amplitude and phase of microwave pulses over a certain frequency range and, therefore, to go beyond the exclusive use of rectangular microwave pulses. This allows to increase and, especially, to shape the excitation profile of microwave pulses for each specific spin system (for recent reviews see [17,18]). Newly designed AWG-based pulse sequences together with the availability of spin labels spectroscopically orthogonal to nitroxides yield considerable sensitivity improvements, marking the beginning of a new era in pulsed EPR [19–26]. These novel AWG-based sequences are still facing several challenges, mainly regarding the compensation for resonator and microwave bridge response functions for pulse shape optimization and, connected with that, the suppression of artifacts. The latter is still problematic, for example in CP (Carr-Purcell) DEER [20,27–29]. In case of 4-pulse DEER, as we will see later, an immediate, straightforward way to exploit the advantages of arbitrary waveform generators is the utilization of Gaussian pulses instead of conventional rectangular ones.

*"2+1" pulse train ESE.* The "2+1" pulse train ESE first described by Kurshev et al. [30,31] is a single-frequency experiment that allows the extraction of the dipolar coupling strength between electron spins as in the two-frequency DEER experiment (Fig. 1). In a DEER experiment, if there is an overlap between the pump and observer excitation bands, the spins within the overlap are subject to a "2+1" pulse train experiment [13]. Consequently, the acquired DEER data consist of a superposition of the time traces from both experiments, with the "2+1" pulse train signal emerging as an artifact primarily visible at the end of the DEER dipolar evolution time.

Calculating the expected time evolution of the echo intensity when pump and observer pulses overlap has been addressed for 3-pulse ELDOR by Salikhov [32]. When the frequency $\omega_B$ of the pump pulse is close to the frequency $\omega_A$ of the echo-forming pulses, there is a growing contribution of a new term proportional to $\langle\cos(D(\tau - T))\rangle$, which is responsible for the artifact observed. The angle brackets "$\langle\rangle$" indicate averaging over the orientations of the distance vector and distances between spin probes, $D$ is the dipolar coupling parameter of the $\widehat{A}$-term of the dipolar alphabet. In 4-pulse DEER, $\tau$ would correspond to $d_2$ in Fig. 1.

The $T$-dependence of the DEER echo intensity, proportional to $\langle\cos(DT)\rangle$, is opposite to that of the new term. Therefore, the maximum of the dipolar oscillation is expected at $T = 0$ for the DEER signal and for the artifact at $T = \tau\ (= d_2)$.

Due to the $d_3$ time in 4-pulse DEER (to avoid pulse overlaps, see Fig. 1), we think that the maximum of the new contribution (artifact) cannot be detected. Therefore, for different dipolar frequencies, the position of the last visible oscillation will change (as seen experimentally).

The presence of such an artifact towards the end of the 4-pulse DEER time trace strongly affects the reliability of the background fit of the DEER time trace and the determination of the width of the distance distribution becomes cumbersome, especially if the dipolar oscillation did not completely decay during the available dipolar evolution time. Therefore, as we will see later, removing such interferences can increase the fidelity of interspin distances obtained by DEER.

*Pulse shapes.* AWGs allow the utilization of complex pulse shapes which can optimize the excitation bandwidth for a given spin system. Since the Fourier transform of a rectangular pulse is a sinc function, it might be tempting to use a sinc function as a

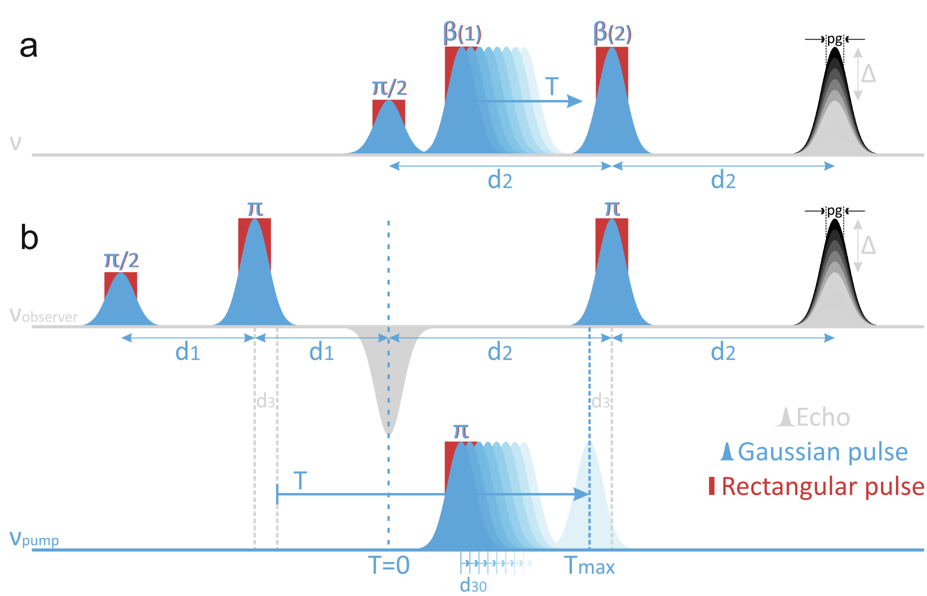


**Fig. 1.** Pulse sequences with conventional rectangular (red) or Gaussian pulses (blue). (a) "2+1" pulse train ESE sequence with β(i) being the flip angles of the pulses. The pump pulse β(1) should be ideally a 2π/3-pulse [31]. The length of the integration window of the refocused echo is marked as pg. Δ highlights the modulation depth and T is the dipolar evolution time. (b) Dead-time free 4-pulse DEER sequence. $T_{max}$ is the maximal experimental dipolar evolution time (d1 + d2-2d3) with d3 being a delay to avoid pulse overlaps and d30 the time step of the pump pulse. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)